THE SOIL ALGA *JAAGIELLA ALPICOLA* VISCHER (CHLOROPHYTA), A NEW MEMBER FOR THE ORDER PLEURASTRALES

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ABSTRACT — *Jaagiella alpicola* Vischer is an edaphic alga that forms filaments and cell packets. The cells have a single nucleus and a plate-like parietal chloroplast without a pyrenoid. A metacentric mitotic spindle was observed during cell division; the parental cell wall contributes to the formation of the common transverse cell wall. Biflagellated zoospores were formed in groups of two or four within non-modified cells of the thallus; the parental cell wall does not contribute to the zoospore cell envelope. The zoospores were naked, almost flattened, globally asymmetric and contained a single chloroplast with an eyespot. The basal bodies showed counter-clockwise orientation and were connected by a striated proximal and distal connecting fibre; the flagellar roots consist of a cruciate (7(6+1)-2-7(6+1)-2) microtubular root system. The ultrastructural and reproductive features of *J. alpicola* suggest its inclusion in the order Pleurastrales.

RÉSUMÉ — *Jaagiella alpicola* Vischer est une algue édaphique qui forme des filaments et des paquets sarcinoïdes. Les cellules présentent un seul noyau et un chloroplaste pariétal en forme de plateau, sans pyrenoïde. Le fuseau mitotique est métacentrique; la paroi de la cellule mère contribue à la formation de la paroi transversale. Les sporocystes sont des cellules du thalle non modifiées, qui donnent deux ou quatre zoospores biflagellées; la paroi de la cellule mère ne contribue pas à la formation de la zoospore. Les zoospores sont nues, plus ou moins comprimées et asymétriques, et ont un seul chloroplaste qui présente un stigma. Les corpuscules basilaires présentent une disposition *counter-clockwise* (interprétée comme résultant d'une dérive dans le sens contraire des aiguilles d'une montre par rapport à une disposition ancestrale hypothétique). Ils sont connectés entre eux par deux fibres striées, l'une proximale et l'autre distale. Les racines flagellaires constituent un système microtubulaire cruciforme (7(6+1)-2-7(6+1)-2). Les caractéristiques morphologiques et ultrastructurales de *J. alpicola* suggèrent l'inclusion de cette espèce dans l'ordre des Pleurastrales.

KEY WORDS: chloroplast division, green algae, *Jaagiella*, Microthamniales, Mitosis, Pleurastrales, ultrastructure, zoospore.

INTRODUCTION

The genus *Jaagiella* (Chlorophyta) was established by Vischer (1960). Bourrelly (1972) placed the genus in the Chaetophoraceae.

The original strain was unfortunately lost (Gärtner, pers. comm.). However, *J. alpicola* Vischer was rediscovered (Vela & Hernández-Mariné, 1987). A morphological and ultrastructural study was done (Hernández-Mariné & Revilla-Estrach, 1989); the habitat, appearance, and the presence of naked and flattened zoospores suggested its classification in the Pleurastrophyceae (Hernández-Mariné & Revilla-Estrach, 1989).

Based on differences in cell division and structures associated with flagellar apparatus, Melkonian & Berns (1983) and O'Kelly & Floyd (1984) suggested a group with distinctive features that was established as a class, the Pleurastrophyceae (Mattox & Stewart 1984), including the orders Pleurastrales (with the genera *Pleurastrum*, *Microthamnion*, *Pseudotrebouxia*, *Trebouxia*, and *Friedmannia*) and Tetraselmidales (with *Tetraselmis* as the only genus). The Pleurastrophyceae was in time expanded with the addition of other taxa, based on both non-molecular and molecular evidence (Deason & Floyd, 1987; Kantz *et al.*, 1990; Zechman *et al.* 1990; Friedl & Zeltner, 1994; Steinkötter *et al.*, 1994; Watanabe & Floyd, 1994; Friedl, 1995, 1996; and a revision by Bakker *et al.*, 1997) whereas the genus *Pleurastrum* was shown to be of multiple origins (polyphyletic) and, therefore, its species were accorded to different classes (Friedl, 1996). Moreover, the order Pleurastrales was proposed as order Microthamniales by Melkonian (1990) due to the relatively unspecialized zoospores in *Microthamnion*. Lacking the latin diagnosis, the Microthamniales has not been validly published, althought it has been adopted for most of the specialists of this group (Friedl & Zeltner, 1994; Steinköter *et al.*, 1994; Bakker *et al.*, 1997). The order Pleurastrales was included in the class Ulvophyceae, because of the same basic flagellate-type apparatus (Sluiman, 1989). Combining molecular and non-molecular data, Kantz *et al.* (1990) found that the Pleurastrophyceae have a closer relationship with the Chlorophyceae than with the Ulvophyceae, although both possess the counter-clockwise basal body orientation in flagellated cells. Ribosomal DNA sequence data suggest the monophyletic origin of the Pleurastrales (as Microthamniales), and as it forms an independent evolutionary lineage together with autosporic coccoid green algae, it was suggested to treat it as a separate class of green algae (Friedl, 1995). The name Trebouxiophyceae was chosen for this class since the type species of the genus *Pleurastrum* was found as a member of the Chlorophyceae and because lichen algae, such as *Trebouxia impressa* V. Ahmadjian may best represent this class (Friedl, 1995). The independence of the Trebouxiophyceae seems clear and it is accepted that ultrastructural features of the zoospore and molecular data are both reliable and comparable for assessing phylogenetic relationships (Friedl & Zeltner, 1994; Lokhorst & Rongen, 1994; Friedl, 1995; Bakker et al., 1997). The purpose of this investigation was to obtain more information on non-molecular features of the monospecific genus *Jaagiella* and contrast the structural and ultrastructural data with recent information on the Pleurastrales.

MATERIAL AND METHODS

Isolates of *J. alpicola*, derived from enrichment culture from Cap Norfeu (Vela & Hernández-Mariné, 1987) were used. Cultures were grown either in liquid or on 2% agarized Bold Basal medium (BBM) (Nichols & Bold, 1965), at different pH values under a 12:12h light/dark regime, 15-27° C and a light intensity of 40-100 $\mu E\ m^{-2}\ s^{-1}$. Zoospore production was attempted by doubling the concentration of phosphorous (K_2HPO_4) in the BBM or by changing pH, temperature and light regimes. Material from young colonies

or groups with freshly liberated zoospores was collected depending on the type of cell to be studied, namely vegetative cells or zoospores.

A Nikon Optiphot light microscope and a NIKON FX-35DX photographic machine were used. For transmission electron microscopy two fixation methods were followed: (1) chemical fixation and (2) cryofixation followed by cryosubstitution. For chemical fixation, the samples were fixed with glutaraldehyde (2%) in cacodylate buffer for two hours at 4° C, washed in the same buffer, postfixed in 2% OsO_4 (in cacodylate buffer) for 90 min. at 4° C, and rinsed in the same buffer. Part of the chemically fixed material was critical point dried and observed under the scanning electron microscope (SEM). Cryofixation was performed by projection against a Cryoblock (Reichert-Jung) cooled with liquid nitrogen (-196° C) according to Escaig (1982). After cryofixation the samples were cryosubstituted at -90° C for three days in a homemade cryosystem (Quintana, 1994) using acetone with 1-3% osmium tetroxide and then processed as described by Porta & López-Iglésias (1998). For freeze-fracture, filaments were frozen in Freon. Freeze-fracturing was carried out in a Balzers 301 high vacuum freeze-etch unit (Balzers A6, Balzers, Lichtenstein) and the specimen was shadowed with platinum and coated with carbon. Three electron microscopes were used: 1) Philips EM 200, 2) HITACHI H800MT, and 3) HITACHI 600 scanning electron microscope.

RESULTS

On solid BBM the heterotrichous thalli of *Jaagiella alpicola* developed into colonies consisting of richly branched filaments radiating out from irregularly sarcinoid packets (Fig. 1). The outer filaments were openly branched and consisted of cylindrical and sometimes slightly elongated cells, especially at apices. The sarcinoid packets were formed of subglobose cells. A ball-like mass of filaments was formed when cultured in liquid media.

The filaments and sarcinoid packets were covered by a thin gelatinous matrix. Each vegetative cell contained a single, medium size, plate-like parietal chloroplast lacking a pyrenoid. Scattered starch granules were present. Pyrenoglobuly were lacking. A large subspherical nucleus was usually located next to the chloroplast. Centrioles were never seen in the interphase of vegetative cells. Large vacuoles containing electron dense material and multivesicular bodies were sometimes visible. The cell wall had smooth contours in the young cells. During maturation some undulations appeared on the cell membrane, along the whole cell perimeter (Figs 2, 3). Sexual reproduction was not observed. Asexual reproduction occurred by fragmentation and by zoospores. The spherical innermost vegetative cells of the sarcinoid packets were the first to undergo zoosporogenesis, with no visible changes in size or shape (Fig. 4). Terminal cells sometimes remained in the vegetative condition despite the transformation of adjacent cells into a zoosporangium. The first sign of zoosporulation was cytoplasm separation from the cell wall, leaving an electron transparent fibrillar network. 2-4 zoospores were liberated by the rupture of the parent cell wall. No protocol was effective in inducing the production and release of the zoospores. Sometimes zoospores were produced after introducing an ageing culture into a fresh medium. Zoosporulation mainly occurred in spring, although having been grown in controlled culture conditions for fourteen years.

Upon cessation of motility the zoospores became spherical. Germination of

zoospores was unipolar, although they developed bipolarly soon afterwards. Two weeks after zoospore settlement the filaments were composed of 8-28 cells and sarcinoid packets were developed; intercalary cells were almost 9 µm long and 7 µm wide, shorter than the apical cells, which were about 14 µm in length and 7 µm in width. In ageing cultures, intercalary cells were globular and around 8 µm in diameter.

Zoospores were about 4-6 µm in length, naked, almost pyriform and bore two equal flagella. They were asymmetric; the surface of the ventral face was slightly concave, whereas the dorsal face was convex (Fig. 5). The zoospores were uninucleate. The nucleus was located in the anterior portion of the zoospore, close to the basal bodies. A simple, large, parietal chloroplast filled the posterior area. An extended A-type eyespot (Dodge, 1973) on the dorsal posterior side of the cell; it contained nearly 50 regular electron-dense hexagonal lipid globules, situated in the same plane. It lay beneath the chloroplast envelope membranes, causing a slight bulge at the edge of the chloroplast (Fig. 6). A pyrenoid was lacking. A single mitochondrion with lobes parallel to the ventral side of the cell extended from basal bodies to the surrounding chloroplast. Several membrane-containing vesicles were situated near the basal body. The equal flagella were naked. The axoneme ended in a hair-point (Fig. 5). The transition region (tr) was separated in two unequal parts by the transverse septum or diaphragm (D) (Figs 7, 8): a proximal (ptr) and a distal (dtr) region; the dtr region being twice bigger than the ptr one. The D was associated with both the dtr and ptr (Figs 7, 8). The basal body (bb) (calculated according to Melkonian, 1984), was around 300-500 nm long (Fig. 8). At the end of the cartwheel structure, the bb had a thickened electron-dense plate perpendicular to the axis of the flagella (Fig. 8) that closed the proximal end of each basal body. Two striated connecting fibres were present between the two bb (Fig. 9); the distal striated connecting fibre (dcf) wrapped them externally; the proximal striated connecting fibre (pcf) tied both bb next to their inner angle and was in contact with three triplets of each bb (Fig. 9). According to the basal body absolute orientation system (O'Kelly & Floyd, 1983), the bb of the two flagella showed counter-clockwise orientation (Fig. 10) or an 11-5 o'clock configuration (Fig. 11) (Melkonian & Berns, 1983; Mattox & Stewart, 1984). An X-2-X-2 cruciate microtubular flagellar root system (Moestrup, 1978) was present. The X roots, located on the inner side of the overlapping basal bodies, were constituted by 6+1 microtubules, the single one being in the innermost position. The R-2 roots are located on the outer side of each basal body (Figs 11, 12).

In vegetative cells, the chloroplast divided by an asymmetric constriction. Mitochondria, vacuoles containing medium electron-dense material and some microtubules (arranged parallel to the division plane) were in the area of constriction (Figs 13, 14). Ingrowth of the cell wall and nuclear division apparently took place immediately after chloroplast division. The plane of cell division was parallel to the chloroplast division plane. The centrioles were visible from preprophase. During prophase, centrioles apparently duplicated, the nuclear envelope disappeared, and an asymmetric cell membrane invagination was apparent at opposite poles of the cell; this invagination was larger at the pole containing the centrioles. During metaphase (Fig. 15), the duplicated centrioles were located in pairs on both sides of the cleavage furrow, which was marked by the development of the cell membrane invaginations, in the same plane as the chromosomes (a metacentric spindle). A network of microtubules was formed radiating out of the chromosomes in a plane perpendicular to the centrioles (Fig. 15). At the end of the division process the nuclear envelope was reconstituted around the two daughter nuclei, each located beside the cleavage furrow (Fig. 16).

DISCUSSION

The morphological features of *Jaagiella alpicola* observed in the present study were consistent with Vischer's (1960) original description. Zoosporulation, not described by Vischer, was present. We did not observe akinetes, although according to Vischer's drawings, they could be mistaken for settled zoospores.

Zoospore ultrastructure is consistent with that in other genera of the Pleurastrales. The shape of *J. alpicola* zoospores was asymmetric as in *Pseudotrebouxia* (Melkonian & Peveling, 1988). Like *Leptosira terrestris* Printz (as *Pleurastrum terrestre* Fritsch *et* John in Melkonian, 1981), it had a type A eyespot (Dodge, 1973). It also showed a little bulge at the eyespot belt as is described for *Microthamnion* (Watson & Arnott, 1973; Watson, 1975). The electron dense structure at the basal body basement was similar to a structure described elsewhere; it has been called either a "flagellar platform" in *Microthamnion* (Watson, 1975) or a "terminal cap" in *Myrmecia israeliensis* (Chantan. *et* H.C.Bold) Friedl (as *Friedmannia israelensis* Chantan. *et* H.C.Bold *in* Melkonian & Berns, 1983). The TR in *J. alpicola* is similar to that in *Pleurastrosarcina* (Deason & Floyd, 1987) since the transverse septum was associated with both the dTR and pTR, and also to that of *Myrmecia israeliens* (as *Friedmannia israelensis*, Melkonian & Berns, 1983) and *Microthamnion* (Watson, 1975), although in these two latter cases the D was attached to only one part of the TR. A system II fibre (Melkonian, 1980) forming a rhizoplast in *Microthamnion* (Watson, 1975) and in *Pleurastrosarcina* (Deason & Floyd, 1987) were not observed in *Jaagiella alpicola*. However, as was previously reported for other Pleurastralean genera (Deason & Floyd, 1987; Melkonian & Peveling, 1988), small deviations in zoospore ultrastructure are not considered taxonomically important.

The division process in vegetative cells of *J. alpicola* was consistent with that of some species of the Microthamniales. *J. alpicola* had a typical filament-type chloroplast division apparatus (Tewinkel & Volkmann, 1987), similar to that described for *Trebouxia* (Chida & Ueda, 1991). However, this process was not synchronous with nuclear division but was followed by it.

The vegetative interphase cells that developed during thallus growth, did not have centrioles appearing at the onset of cell division in the same position as in *Leptosira erumpens* (Deason *et* H.C.Bold) Lukešová (Lokhorst & Rongen, 1994). In addition, the parental cell wall contributed to the formation of the common transverse cell wall. This division pattern is considered to be vegetative cell division (Ettl, 1988; Sluiman *et al.*, 1989) rather than "Sporulation" (*loc.cit.*). Moreover, the beginning of the cell division was different depending on the end product. In the process that ended in zoospore formation the parental cell wall did not contribute to the zoospore cell envelope. Thus both cytokinetic events were present in *J.alpicola*, as they are in *L. erumpens* and *Microthamnion* (Lokhorst & Rongen, 1994; Bakker *et al.*, 1997).

Because the present specimen of *J. alpicola* possesses a metacentric spindle and two flagella arranged in a counter-clokwise orientation, with two alternating types of microtubular roots in a cruciate pattern, it was included in the ultrastructurally coherent order Pleurastrales. Since we do not have molecular data, we have been unable to include it into the class Trebouxiophyceae *sensu* Friedl (1995).

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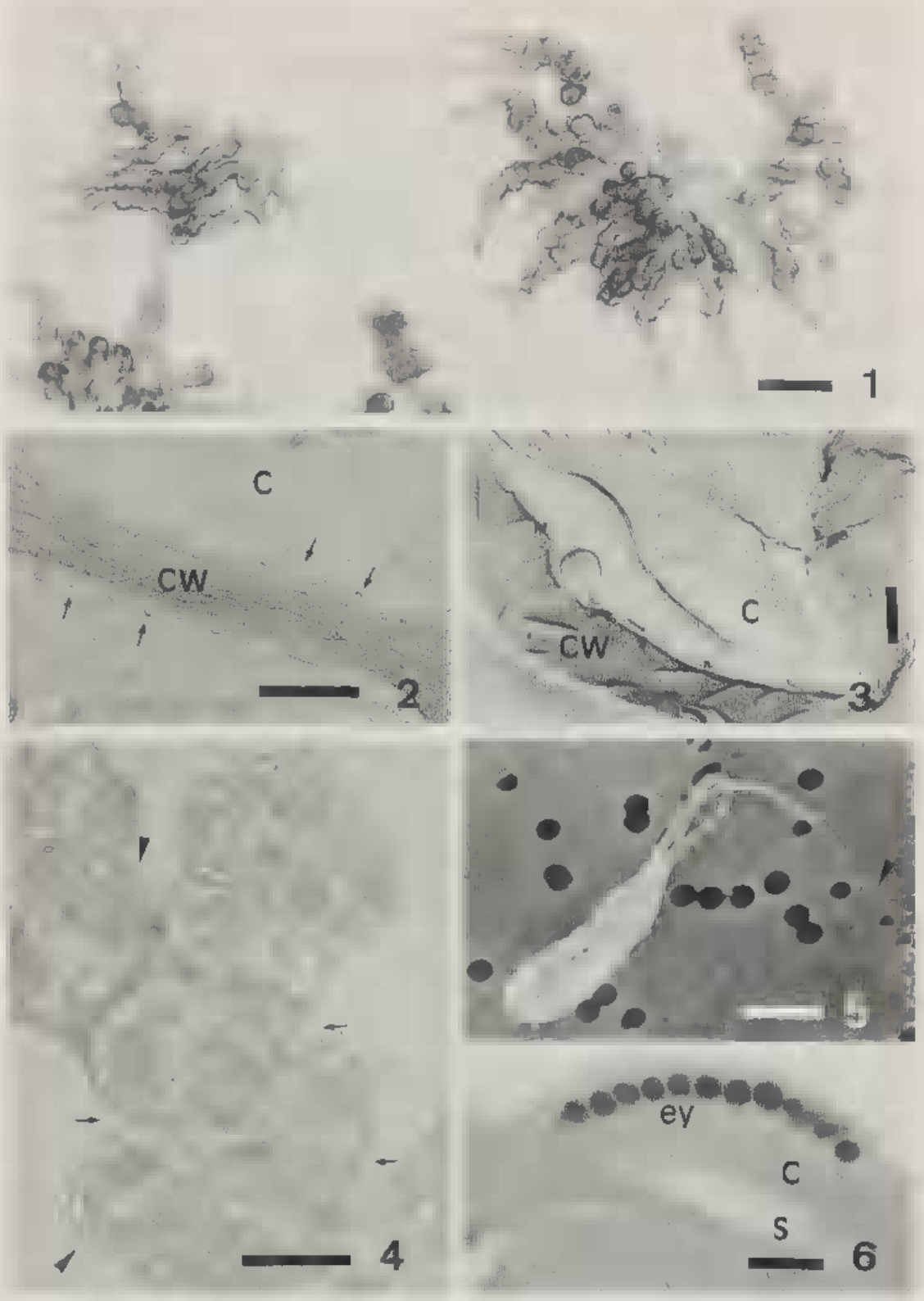
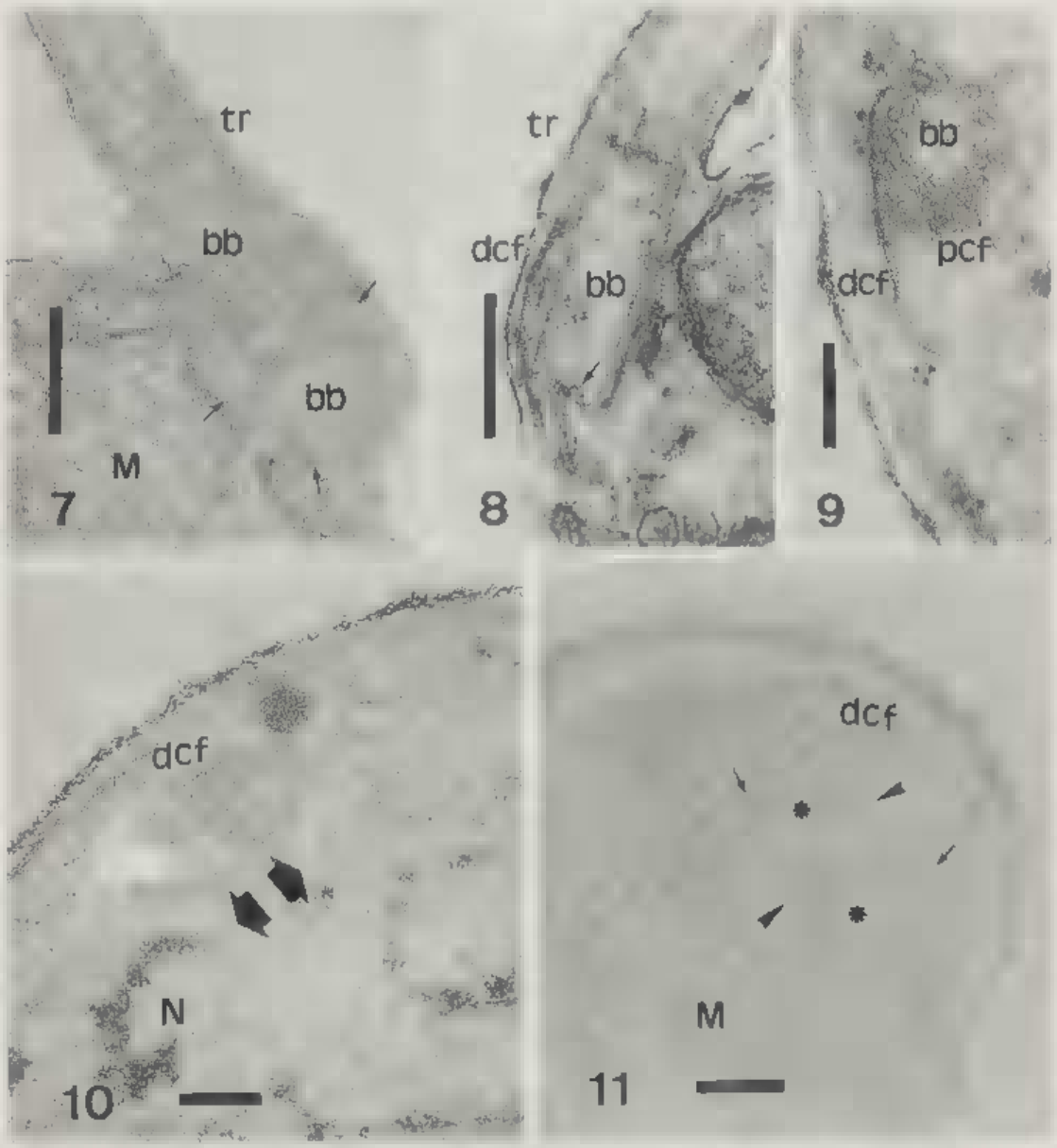


Figs 1-6. Fig. 1. Light microscopy. Thallus formed by filaments and dense sarcinoid packets. Scale bar = 10 µm. Fig. 2. Cell wall of a vegetative cell. Undulations on the cell membrane (arrows). Scale bar = 0.5 µm. Fig. 3. Freeze-fracture of ■ vegetative cell showing the tracks on the cell wall. Scale bar = 0.3 µm. Fig. 4. View of the thallus with cells developed into zoosporangia, revealing four zoospores inside each. Cells are dividing without ingrowth of the cell wall (arrowheads). Note the gelatinous matrix covering the thallus (arrows). Scale bar 5 µm. Fig. 5. SEM view of a free zoospore. The flagella ended in a hair-point (arrowhead). Scale bar 2 µm. Fig. 6. Transverse section in the eyespot region. Notice slight bulge in the plasma membrane. Scale bar = 0.25 µm.
C, chloroplast; CW, cell wall; ey, eyespot; S, starch granules.



Figs 7-11. Zoospore flagellar apparatus. Fig. 7. Anterior end of a zoospore section. Notice the flagellar root microtubules (arrows). Scale bar = 0.25 μm. Fig. 8. Longitudinal section of the proximal region. The transition region, the basal body, the distal connecting fibre and the flagellar platform (arrow) are visible. Scale bar = 0.25 μm. Fig. 9. Transverse section through a basal body showing the proximal and distal striated connecting fibers. Scale bar = 0.25 μm. Fig. 10. Transverse section through the basal bodies showing its counter-clockwise orientation (arrows indicated the arrangement of the triplets of each basal body). Note also the distal striated connecting fibre. Scale bar = 0.25 μm. Fig. 11. Nearly longitudinal section through the basal bodies (asterisks). The flagellar roots are shown: R-X roots (arrowheads), R-2 roots (arrows). Scale bar = 0.25 μm.
bb, basal body; dcf, distal connecting fibre; M, mitochondria; N, nucleus; pcf, proximal connecting fibre; tr, flagellar transition region.

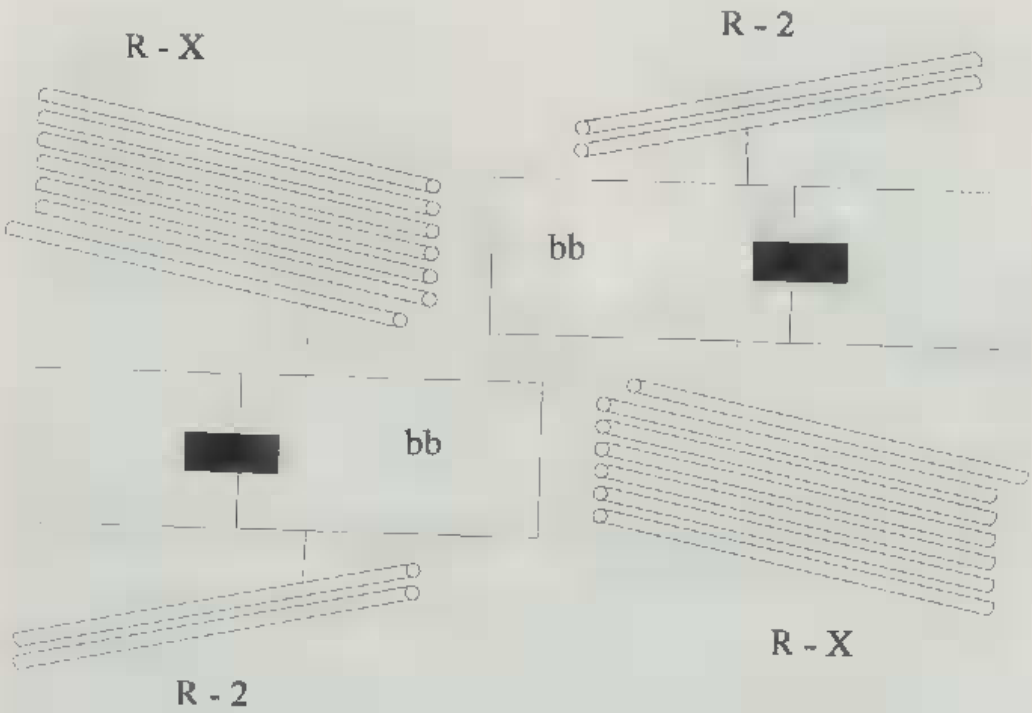
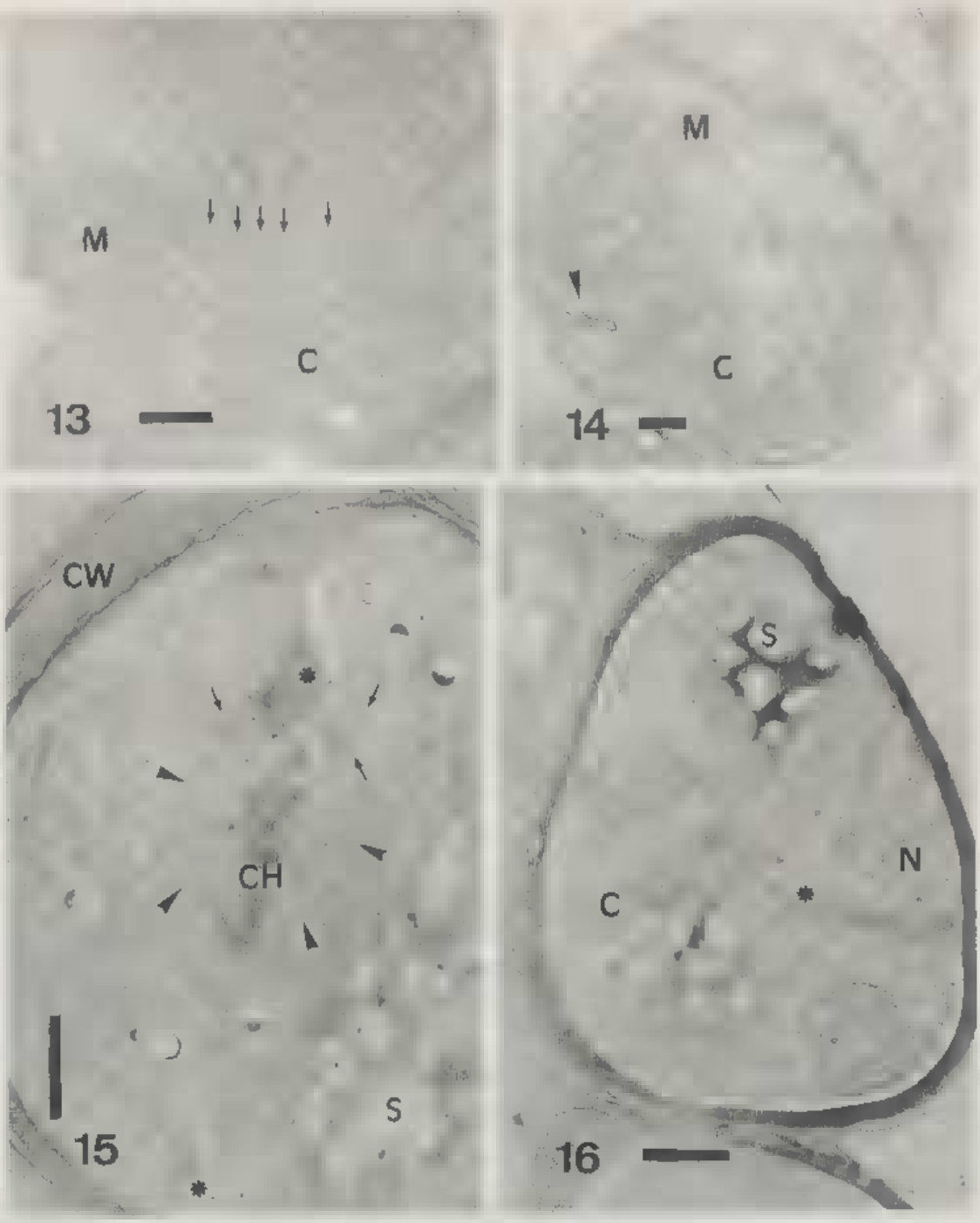


Fig. 12. Schematic drawing of the overlapping basal bodies and the flagellar roots. bb, basal body.



Figs 13-16. Division processes of the vegetative cells. Fig. 13. Isthmus region in a dividing chloroplast. Arrows indicate the transverse microtubules. Scale bar = 0.5 µm. Fig. 14. Vegetative cell at the end of a chloroplast division. Note the initial cell division process indicated by the presence of a infurrowing septum (arrowhead). Scale bar = 1 µm. Fig. 15. Section of a dividing cell at metaphase to show the centrioles (small arrows), the chromosomes, the microtubules of the mitotic spindle (arrowheads) and the infurrowing septum (asterisks). Scale bar = 1 µm. Fig. 16. Cytokinesis. The cleavage furrow (asterisk) was completed. Scale bar = 1 µm.

C, chloroplast; CH, chromosomes; CW, cell wall; M, mitochondria; N, nucleus; S, starch granules.